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Poly(ADP-Ribose)Polymerase-1 (PARP1) Controls Adipogenic Gene Expression and Adipocyte Function

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Poly(ADP-ribose)polymerase-1 (PARP1) is a chromatin-associated enzyme that was described to affect chromatin compaction. Previous reports suggested a dynamic modulation of the chromatin landscape during adipocyte differentiation. We thus hypothesized that PARP1 plays an important transcriptional role in adipogenesis and metabolism and therefore used adipocyte development and function as a model to elucidate the molecular action of PARP1 in obesity-related diseases. Our results show that PARP1-dependent ADP-ribose polymer (PAR) formation increases during adipocyte development and, at late time points of adipogenesis, is involved in the sustained expression of *PPARγ2* and of *PPARγ2* target genes. During adipogenesis, PARP1 was recruited to *PPARγ2* target genes such as *CD36* or *aP2* in a PAR-dependent manner. Our results also reveal a PAR-dependent decrease in repressory histone marks (*e.g.* H3K9me3) and an increase in stimulatory marks (*e.g.* H3K4me3) at the *PPARγ2* promoter, suggesting that PARP1 may exert its regulatory function during adipogenesis by altering histone marks. Interestingly, activation of PARP1 enzymatic activity was prevented with a topoisomerase II inhibitor. These data hint at topoisomerase II-dependent, transient, site-specific double-strand DNA breaks as the cause for poly(ADP)-ribose formation, adipogenic gene expression, and adipocyte function. Together, our study identifies PARP1 as a critical regulator of *PPARγ2*-dependent gene expression with implications in adipocyte function and obesity-related disease models. **(*Molecular Endocrinology* 26: 79–86, 2012)**

Obesity is a complex metabolic disorder characterized by an excess of body fat that is closely associated with other serious health conditions, such as heart disease and diabetes. White adipose tissue, which is the predominant type of fat in adult humans, serves as a storage depot for excess energy. Adipose tissue secretes diverse adipokines, such as leptin, *Adiponectin*, IL-6, TNFα, and others, that regulate blood pressure, immune function, and energy balance (1, 2). Disturbed production of these factors may contribute to the development of insulin resistance or impaired insulin secretion resulting in type 2 diabetes.

During adipogenesis, fibroblast-like preadipocytes differentiate into lipid-laden and insulin-responsive adipocytes, which requires a coordinate up-regulation of many enzymes involved in fatty acid metabolism and pyridine nucleotides as coenzymes (3). The murine 3T3-L1 cell line (4) has been broadly used as a model system to study adipogenesis. 3T3-L1 cells undergo a synchronous differentiation process upon addition of an adipogenic cocktail and have been influential for the understanding of the complex regulatory networks during adipogenesis. Adipocyte differentiation is accompanied by large-scale chromatin changes and by the establishment of transcription factor "hotspots" (5). This process occurs in several stages and involves a cascade of transcription factors, among which the CCAAT/enhancer-binding proteins (C/EBP) C/EBP-β and C/EBP-δ are considered crucial determinants for the expression of the key factor peroxisome proliferator-activated receptor γ (PPARγ) and of C/EBP-α (6, 7). A positive feedback loop between PPARγ and C/EBP-α mutually regulates

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Abbreviations: C/EBP, CCAAT/enhancer-binding protein; ChIP, chromatin immunoprecipitation; NAD^+, nicotinamide adenine dinucleotide; PAR, polymers of ADP-ribose; PARP1, poly(ADP-ribose)polymerase-1; PPARγ, peroxisome proliferator-activated receptor γ; shRNA, short hairpin RNA.

their expression, and together, these two proteins coordinate adipocyte function (7, 8). In contrast to PPARγ1, PPARγ2 expression is mainly restricted to adipocytes (9). PPARγ2, together with several coactivators, regulates an array of target genes, of which *aP2*, *CD36*, and *Adiponectin* have crucial roles for adipocyte function by regulating fatty acid binding, translocation, and catabolism, respectively (5). Deletion of *PPARγ2* in mouse adipose tissues protects against high-fat diet-induced obesity and insulin resistance, but these mice display increased lipid deposition in muscle and liver (10). Similarly, ablation of *PPARγ2* in mice of the ob/ob background results in decreased fat mass, severe insulin resistance, β-cell failure, and dyslipidemia (11).

Poly(ADP-ribose)polymerase-1 (PARP1) (recently renamed ADP-ribosyltransferase diphtheria toxin-like 1) (12) is an abundant, ubiquitous chromatin-associated enzyme that catalyzes the nicotinamide adenine dinucleotide (NAD^+)-dependent addition of polymers of ADP-ribose (PAR) onto a variety of target proteins (13). PAR is a large, negatively charged polymer that functions as posttranslational protein modification. Most PAR in the cell is produced by the catalytic activity of PARP1, which is the main ADP-ribosyltransferase in the nucleus (14). *In vitro*, PARP1 enzymatic activity is strongly activated upon binding to various interaction partners, such as nucleosomes or certain forms of DNA (15). *In vivo*, polymer formation by PARP1 is undetectable by immunofluorescence under basal (unstimulated) conditions but can be detected upon treatment of cells with DNA-damaging compounds (13). Lack of PARP1 in cells was reported to affect the gene expression profile in a genome-wide manner (15). Besides restructuring the chromatin and influencing transcription indirectly (16, 17), PARP1 can additionally act as a transcriptional cofactor during transcription by directly interacting with a variety of transcription factors (18).

Previous studies reported the body weight of adult *PARP1*−/− mice in diverse genetic backgrounds to be different than the corresponding controls (19, 20). Here, we extend these studies to the molecular level and show with different experimental approaches that PARP1-dependent PAR formation increases during adipocyte development and is involved in regulating adipocyte development and function.

Results

ADP-ribosylation is required for sustained PPARγ2-dependent gene expression

To investigate whether PARP1 and its enzymatic activity have an effect on adipogenesis, 3T3-L1 preadipocytes were differentiated into adipocytes by addition of an adipogenic cocktail (21). Western blot analysis from the differentiated 3T3-L1 cell extracts revealed a shift of PARP1 at d 7, suggesting auto-ADP-ribosylation of PARP1 (Fig. 1A). Further Western blot analysis using an antibody against the PAR confirmed that PAR formation was indeed activated after d 3 of adipocyte differentiation. Moreover, PAR formation was strongly reduced in differentiating 3T3-L1 cells that were treated with the PARP inhibitor PJ34 (or Olaparib, data not shown), providing further evidence that ADP-ribosylation was indeed induced in 3T3-L1 extracts (Fig. 1A). A detailed kinetic analysis detected PAR formation already at d 4 after induction of adipocyte differentiation, which remained elevated until d 10 (Supplemental Fig. 1A, published on The Endocrine Society's Journals Online web site at http://mend.endojournals.org).

Interestingly, PJ34 treatment also inhibited storage of free fatty acids in the adipocytes as judged by Oil-Red O staining (Fig. 1B and Supplemental Fig. 1B), suggesting that adipocyte function might require PAR formation. Next, we evaluated the functional contribution of PAR formation for the transcriptional activation of the main

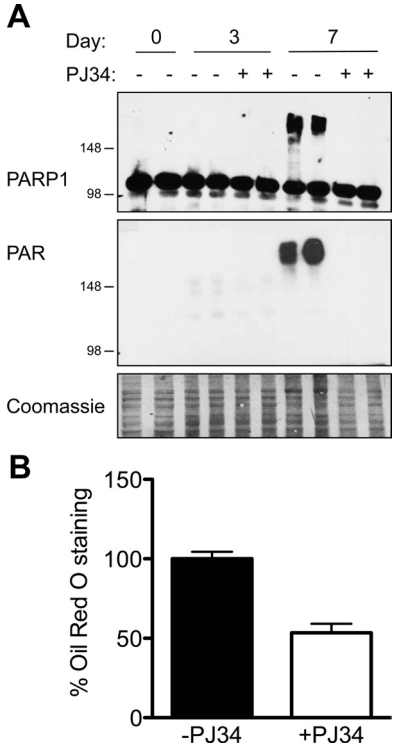

FIG. 1. PAR formation is important during adipogenesis. 3T3-L1 preadipocytes were subjected to adipocyte differentiation during 7 d in the absence or presence of PARP inhibitor PJ34 (10 μM). Inhibitor was supplemented daily. A, Total-cell extracts were prepared and analyzed by Western blotting. An experiment done in duplicates is shown. B, Cells were stained with Oil-Red O, bound Oil-Red O was extracted, and quantified colorimetrically. Result show the mean ± SEM, n = 3.

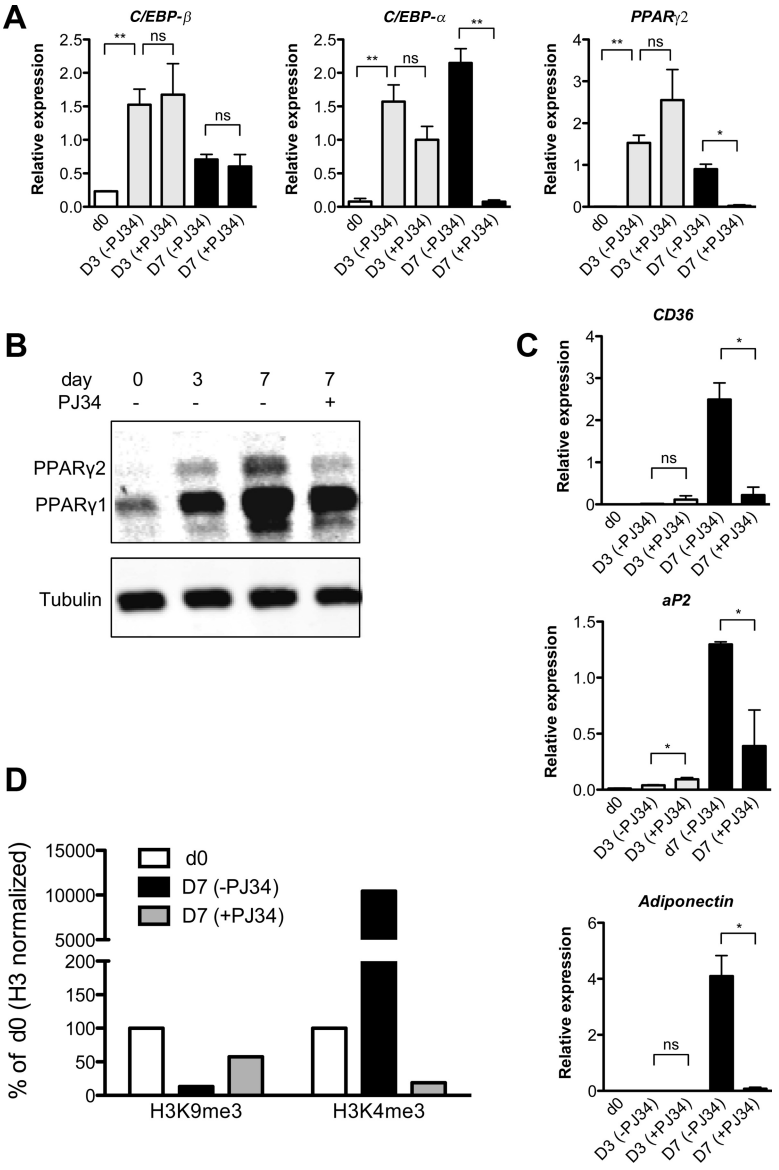

FIG. 2. PAR formation is required for sustained PPARγ2-dependent gene expression. 3T3-L1 preadipocytes were subjected to adipocyte differentiation for 7 d in the absence or presence of PARP inhibitor PJ34 (10 μM). Inhibitor was supplemented daily. A, Real-time RT-PCR analysis for *C/EBP-β*, *C/EBP-α*, and *PPARγ2* is shown. mRNA levels were normalized with *Cyclophilin A*. Results show the mean ± SEM, n = 3. ns, $P > 0.05$; *, $P < 0.05$; **, $P < 001$. B, Total-cell extracts were prepared and analyzed by Western blotting. C, Relative expression levels of the PPARγ2-dependent genes *CD36*, *aP2*, and *Adiponectin* were determined as in A. D, ChIP analysis with H3K9me3 and H3K4me3 antibodies on the *PPARγ2* promoter. The experiment was performed twice with similar results, and one experiment is shown.

drivers of adipogenesis by quantitative PCR. The expression of *C/EBP-β*, the main transcriptional regulator of initial *PPARγ2* expression, as well as of *C/EBP-α* and *PPARγ2*, was significantly induced upon initiation of adipogenesis and not affected by the PARP inhibitor PJ34 (at d 3) (Fig. 2A). In contrast, although the sustained expression level of *C/EBP-β* at d 7 was not affected by PARP inhibition, the *C/EBP-α* and *PPARγ2* mRNA levels at this later time point were significantly reduced by the presence of PJ34 for 6 d (Fig. 2A). The reduction in *PPARγ2* mRNA levels was also manifested in PPARγ2 protein levels, although a residual amount of PPARγ2 was still observed after 6 d of PJ34 treatment (Fig. 2B). Thus, the sustained expression (after d 3 of adipogenesis) of *C/EBP-α* and *PPARγ2* requires the formation and presence of PAR. To elucidate whether the expression of PPARγ2 target genes might consequently also be affected by ADP-ribosylation, we analyzed the expression of *aP2*, *CD36*, and *Adiponectin*. Although *aP2* expression was detected at d 3 and then increased substantially until the late phase of adipogenesis (d 7), *CD36* or *Adiponectin* were significantly expressed only at d 7 (Fig. 2C). Remarkably, the expression of all three genes was abrogated by the PARP inhibitor PJ34 (Fig. 2C), suggesting that PPARγ2-dependent gene expression is indeed regulated by PAR formation.

To investigate whether known histone modifications (22, 23) were affected by PJ34 treatment, repressory (H3K9me3) and stimulatory marks (H3K4me3) were assessed at the *PPARγ2* promoter. Chromatin immunoprecipitation (ChIP) experiments revealed that the repressory histone mark H3K9me3 decreased during differentiation (d 7), whereas PJ34 treatment blocked the release of this repressory histone mark at the *PPARγ2* promoter (Fig. 2D). In contrast, the stimulatory histone mark H3K4me3 increased at the same promoter during adipocyte differentiation (d 7), and PJ34 treatment blocked its accumulation, which is in agreement with the effects that were observed at the gene expression level (Fig. 2D).

3T3-L1 adipocyte differentiation is inhibited by PARP1 depletion

To further assess a specific involvement of PARP1 (but not other PARP family members) and its enzymatic activity in PPARγ2-dependent gene expression, undifferentiated 3T3-L1 preadipocytes were transduced with a lentivirus expressing short hairpin RNA (shRNA) against *PARP1* (to stably knockdown PARP1) or a scrambled

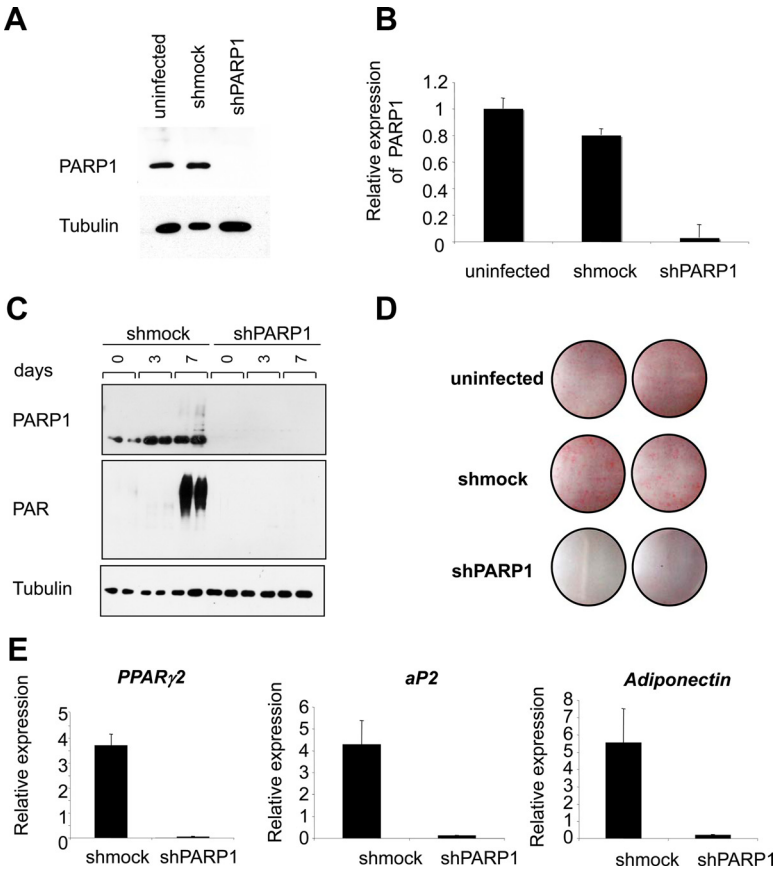

FIG. 3. Depletion of PARP1 retards 3T3-L1 differentiation into adipocytes. Knockdown of PARP1 was confirmed by (A) Western blot and (B) quantitative-PCR analyses. C, shmock or shPARP1 3T3-L1 preadipocytes were differentiated into adipocytes for 7 d. Western blot analysis with total-cell extracts shows PARP1 and PAR levels in shmock and shPARP1 cells during adipogenesis (d 0, 3, and 7 of differentiation). D, Oil-Red O staining of shmock and shPARP1 cultures that were subjected to 7 d of adipocyte differentiation. E, Real-time RT-PCR analysis of *PPARγ2*, *aP2*, and *Adiponectin* in shmock and shPARP1 cells after 7 d of adipocyte differentiation. mRNA levels were normalized with *Cyclophilin A*. Results show the mean ± SEM, n = 3.

control RNA (Fig. 3, A and B). As compared to uninfected cells, *PARP1* mRNA levels decreased slightly in mock-treated cells, whereas in shPARP1 3T3-L1 cells, PARP1 mRNA and protein expression were below 1% of the levels observed in shmock cells (Fig. 3, A and B). Furthermore, no PAR formation was detected in the extracts of differentiated shPARP1 cells (Fig. 3C), suggesting that the observed PAR formation during adipogenesis was indeed dependent on PARP1. Next, we investigated whether depletion of PARP1 affects adipocyte differentiation. As judged by Oil-Red O staining, cells efficiently depleted of PARP1 were strongly hampered in their development to mature adipocytes (Fig. 3D). Moreover, the expression of *PPARγ2* itself and the expression of PPARγ2 target genes such as *aP2* and *Adiponectin* was strongly reduced after 7 d of induced differentiation in shPARP1 3T3-L1 cells (Fig. 3E). These results suggest that PARP1 and PARP1-induced PAR formation are required for *PPARγ2* expression and subsequent adipocyte differentiation and that other PARP family members cannot compensate for the lack of PARP1 during adipocyte differentiation.

Topoisomerase II inhibitor merbarone inhibits PAR formation and PPARγ2-dependent gene expression

Activation of PARP1 and subsequent induction of transcription can be caused by topoisomerase II-mediated, transient, site-specific DNA double-strand breaks (24). We therefore sought to test whether the upstream event that activates PARP1 enzymatic activity requires topoisomerase II-dependent double-strand DNA break formation. To investigate this possibility, 3T3-L1 cells were treated during the last 2 or 3 d of differentiation (treatment at d 5–6 or 4–6, respectively) with PARP inhibitor (PJ34) or topoisomerase II inhibitor (merbarone). PJ34 efficiently inhibited PAR formation even when added only in the later stages of differentiation (d 5 and 6) (Fig. 4A) and could still strongly affect PPARγ2-dependent gene expression, whereas PPARγ2, once expressed, was minimally changed (Fig. 4B and Supplemental Fig. 2, A and B). Interestingly, merbarone also reduced PAR formation significantly, indicating that topoisomerase II activity may be at least partly responsible for PARP1 activation.

In addition, the expression of *PPARγ2*, *aP2*, *Adiponectin*, and *CD36* was strongly reduced in cells treated with merbarone during d 5 and 6 (Fig. 4C). To rule out that merbarone directly inhibits PARP1 enzymatic activity, we performed *in vitro* auto-ribosylation assays (Supplemental Fig. 2C). Although PJ34 efficiently blocked PAR formation, merbarone did not have an *in vitro* inhibitory effect on PAR formation, even at concentrations as high as 80 μM (Supplemental Fig. 2C). These results suggest that topoisomerase II is responsible for the observed activation of PARP1 during the later phase of adipogenesis. Unfortunately, we were not able to demonstrate recruitment of topoisomerase II to the *CD36* and *aP2* promoters in adipocytes with the available antibodies.

PAR formation enhances PARP1 chromatin recruitment

To further address the direct functional link between PARP1 and PAR formation for PPARγ2-dependent gene

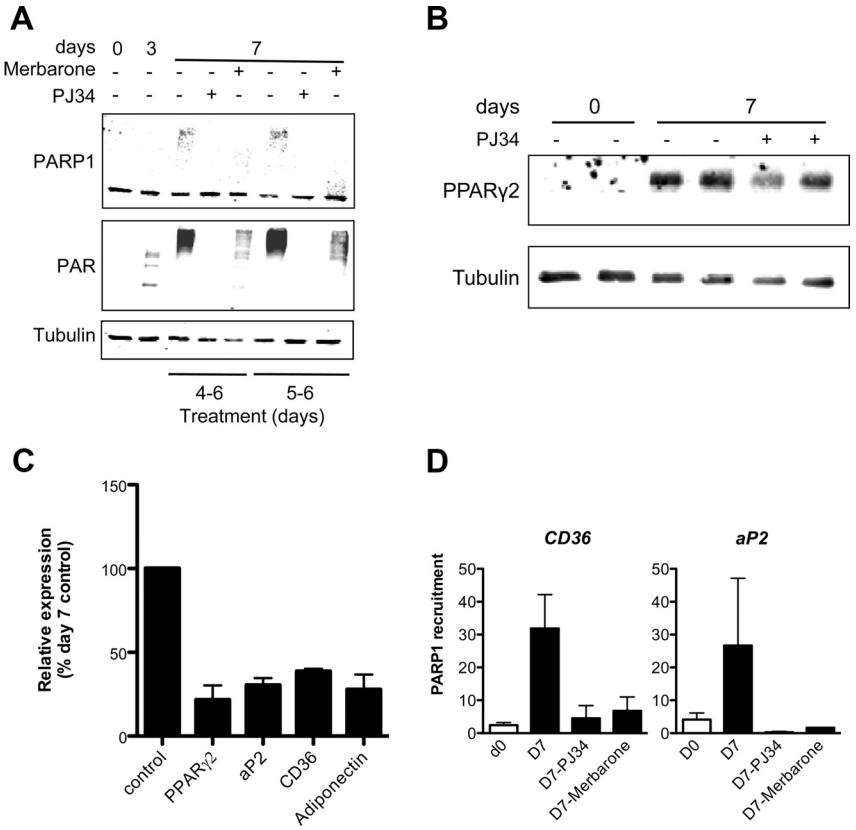

FIG. 4. PAR formation is dependent on topoisomerase II activity and required for PARP1 chromatin recruitment. A, 3T3-L1 preadipocytes were differentiated for 7 d. At d 4–6 or 5–6, 10 μM PJ34 or 40 μM merbarone was added to the medium. Total-cell extracts were prepared and analyzed by Western blotting. B, 3T3-L1 preadipocytes were subjected to adipocyte differentiation. 10 μM PJ34 was supplemented at d 5 and 6. Total-cell extracts from the indicated days were analyzed for PPARγ2 protein expression. C, Real-time RT-PCR analysis on d 7 from differentiated 3T3-L1 cells that were treated at d 5 and 6 of differentiation with dimethyl sulfoxide control or 40 μM merbarone. mRNA levels were normalized with *Cyclophilin A*. Results are from three independent experiments, mean ± SEM. D, ChIP analysis with the PARP1 antibody on *CD36* and *aP2* promoter. Negative control (beads from d 0 samples) was set as 1. Results are from five independent experiments, mean ± SEM.

expression, we investigated the recruitment of PARP1 to PPARγ2 target promoters by ChIP. ChIP analysis revealed that PARP1 was already present at the *CD36* and *aP2* promoters in undifferentiated cells, but this association was substantially increased at d 7 (Fig. 4D). Interestingly, PARP1 recruitment was abrogated when the cells were treated either with PJ34 or with merbarone, suggesting that the association of PARP1 with the tested promoters was dependent on PAR formation, which is induced by topoisomerase II activity.

Discussion

In this study, we provide evidence that PARP1 controls adipocyte function *in vitro*. Our results show that PARP1 is activated after the initial phase of adipogenesis and that either depletion of PARP1 or inhibition of ADP-ribosylation strongly retard the functional differentiation of 3T3-L1 cells. These findings are in agreement with earlier results showing that PAR formation during adipogenesis is increased, although an initial decrease at the beginning of adipocyte differentiation was observed (25). Furthermore, inhibition of topoisomerase II strongly reduced PARP1 activation and adipocyte function.

Recent genome-wide analyses revealed a dramatic and dynamic modulation of the chromatin landscape during the first hours of adipocyte differentiation that coincides with cooperative binding of multiple early transcription factors (including glucocorticoid receptor, retinoid X receptor, *Stat5a*, C/EBP-β, and C/EBP-δ) to transcription factor hotspots (5). C/EBP-β marks a large number of these transcription factor hotspots before induction of differentiation and chromatin remodeling and is required for their establishment. Because the expression of *C/EBP-β* was independent of PAR formation during the whole differentiation period (Fig. 2A), our experiments suggest that PAR formation is not required for the formation of the transcription factor hotspots. In contrast, although the initial induced gene expression of *PPARγ2* and *C/EBP-α* (at d 3) was not affected by PJ34 treatment, the sustained expression of both genes was reduced upon inhibition of PAR formation. Therefore, PAR appears to be necessary for the positive feedback loop that brings about the mutual stimulation of *PPARγ2* and *C/EBP-α* expression. Sustained PPARγ2-dependent gene expression was abrogated even after inhibition of PAR formation only for the last 2 d (d 5 and 6), whereas PPARγ2 protein levels were affected minimally (Fig. 4B). These results provide convincing evidence that the induction of PAR formation and its maintenance are important for sustained PPARγ2-dependent gene expression and adipocyte function. Moreover, this indicates that the reduced PPARγ2 target gene expression is not caused by a lack of cellular PPARγ2 but likely due to an additional function of PAR in PPARγ2-dependent gene expression. Even though the genetic experiments and inhibitor studies demonstrate a strong correlation between PAR formation and adipo-

genic gene expression, additional off-target effects of the PJ34 inhibitor cannot be excluded (*e.g.* on other NAD^+ consuming enzymes). However, given that most of the observed PAR formation at d 7 was associated with PARP1 and that PARP1 knockdown hampered adipogenesis, it is very likely that PARP1 enzymatic activity is required for this process, although we can currently not completely rule out that other ADP-ribosyltransferases, such as PARP2, also contribute to the observed effect (26).

PAR formation by PARP1 could affect adipogenesis in multiple ways: 1) by excluding or retaining transcription factors from a special chromatin site (7); 2) by dissociating corepressors that occupy *PPARγ2* and PPARγ2 target promoters during adipocyte differentiation, allowing the recruitment of transcription coactivators; and finally 3) by regulating histone modifying enzymes and subsequently altering histone modifications (27).

Along this line, mechanistically, our data implicate that topoisomerase II-induced DNA strand breaks as an upstream event preceding PAR formation, whereas altered histone modifications may represent the downstream effect of PARP1 activation. The reduced H3K4 trimethylation (Fig. 2D) upon PJ34 treatment is in agreement with the finding that PARP1 prevents H3K4me3 demethylation (27). In addition, our data further suggest an effect of PARP1 on the H3K9me3 modification (Fig. 2D). PAR could affect H3 marks either via histone demethylases (*e.g.* lysine demethylases) or through specific methyltransferases (*e.g. EHMT1/2*, *GLP*, *SETDB1*, or Suv39h1). Consequently, PAR formation may cause a shift from repressory to activatory histone marks and thereby affect adipogenesis. PARP1 itself is activated by DNA damage, which could be brought about by topoisomerase II activity. Topoisomerase II cuts DNA strands during replication (separation of DNA supercoils). However, the later stages of adipocyte differentiation are accompanied by an exit from the cell cycle (28), thereby excluding this replication-dependent phenomenon as an explanation for the regulation by topoisomerase II. The induction of PARP1 by topoisomerase II in differentiating adipocytes is therefore likely to be mediated by a transient, site-specific double-strand DNA break at the promoters of PPARγ2-dependent target genes, as described for nuclear receptors (29).

PARP1 recruitment to the promoters of different PPARγ-dependent target genes was strongly enhanced upon PAR formation. This observation is in contrast to earlier *in vitro* studies, suggesting that automodified PARP1 is released from chromatin (17) or that PJ34 treatment does not affect PARP1 binding to target gene promoters (27). However, in *Drosophila*, the presence and activity of PARP1 is also required to maintain a transcription compartment by retaining transcription factors (30), which also suggests a function for PARP1 enzyme activity at the promoters of active genes as our findings in this study.

The cellular PAR levels are determined by the synthesizing activities of PARP and the degradation by poly-(ADP-ribose)glycohydrolase, the main cellular enzyme required for PAR degradation. The fact that PAR levels were reduced upon the inhibition of PARP1 shortly before the extraction (data not shown) suggests that these PAR polymers are not stable but rather constantly formed and degraded. Consequently, the strong PAR formation during adipocyte differentiation (as compared with the PAR formation as a response to genotoxic stress such as H_2O_2 treatment) must be due to constant synthesis, which has been documented for the late stages of adipogenesis (25). However, activation of ADP-ribosyltransferases and of PAR formation can drastically affect cellular metabolite levels, such as NAD^+, nicotinamide adenine dinucleotide phosphate, ATP, or glucose-6-phosphate and thereby even indirectly impair cell viability (31). Interestingly, NAD^+ levels during adipogenesis were slightly elevated (Supplemental Fig. 3) or even drastically increased during adipocyte differentiation of 3T3-L1 cells as described by others (3). This explains why the differentiated cells are not dying upon extensive PAR formation (due to the extended NAD^+ and subsequent ATP depletion) as suggested earlier (32). Thus, high NAD^+ levels may maintain PAR formation due to increased substrate availability in a DNA damage-independent manner. In summary, these data suggest that increased NAD^+ synthesis fuels the substantial PAR synthesis and thus allows a constant PAR turnover during adipogenesis.

In conclusion, our data reveal a novel metabolic function of PARP1 in adipose tissue and provide evidence that PARP1 and topoisomerase II regulate the adipogenic gene expression program. These observations additionally provide insight into the link between cellular metabolism (*e.g.* production of NAD^+), gene expression, and differentiation. It will be interesting to investigate whether the inhibition of ADP-ribose formation by PARP inhibitors affects the pathological conditions, such as metabolic disorders.

Materials and Methods

3T3-L1 cell culture and differentiation

3T3-L1 preadipocytes were cultured in DMEM containing 10% (vol/vol) fetal calf serum. On d 2 (2 d after 3T3-L1 prea-

dipocytes reached confluence), cells were induced to differentiate by insulin (5 μg/ml), 3-isobutyl-1-methylxanthine (0.5 mmol/liter), and dexamethasone (1 μmol/liter). On d 4, regular DMEM containing insulin (5 μg/ml) was substituted until d 7. Cells were differentiated in the presence or absence of PJ34 (10 μM), which was supplemented to cells every 24 h. Differentiation was monitored by morphological assessment and Oil-Red O staining. For Oil-Red O staining, cells were washed twice with PBS, fixed in 10% formaldehyde for 1 h, and stained for 10 min with 0.2% (wt/vol) Oil-Red O solution in 60% (vol/vol) isopropanol. Cells were then washed several times with water, and excess water was evaporated by placing the stained cultures at approximately 32 C. For the quantification of the staining, the Oil-Red O was extracted with 100% isopropanol from the cells and measured at 500 nm.

Reagents

Dexamethasone (D-4902), 3-isobutyl-1-methylxanthine (I-5879), insulin (I-9278), Oil-Red O (00625), and merbarone (M2070) were obtained from Sigma-Aldrich (St. Louis, MO). Anti-PARP (sc-7150) antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), anti-PAR (51-8114KC) from BD Pharmingen (San Diego, CA), anti-H3 (1791-100) from Abcam (Cambridge, MA), anti-H3K9me3 (07-442) from Millipore (Bedford, MA), anti-H3K4me3 (07-473) from Millipore, and antitubulin (T6199) antibody from Sigma-Aldrich. PJ34 was purchased from Alexis Biochemicals (San Diego, CA) (ALX-270-289-0000).

Whole-cell extraction

Whole-cell extracts were prepared by lysing the cells for 20 min in radioimmunoprecipitation assay buffer [50 mM Tris (pH 8), 400 mM NaCl, 0.5% Nonidet P-40, 1% deoxycholate, 0.1% sodium dodecyl sulfate, 1 μg/ml pepstatin, 1 μg/ml bestatin, 2 μg/ml leupeptin, 2 mM phenylmethylsulfony lfluoride, 10 mM β-glycerophosphate, 1 mM NaF, and 1 mM dithiothreitol] at 4 C rotating on the wheels. Lysate was centrifuged for 20 min at 4 C at 14,000 rpm. Total proteins were loaded on 7.5% sodium dodecyl sulfate gels and blotted with anti-PAR, anti-PARP, anti-PPARγ, and antitubulin antibodies.

In vitro ADP-ribosylation assay

ADP-ribosylation assays were performed as earlier described (33). Briefly, 400 nM PARP1 was incubated with 100 nM ^{32}P-NAD$^+$ and 200 nM DNA in PARP reaction buffer in the presence or absence of PJ34 (20 μM) or merbarone (20, 60, or 80 μM).

NAD measurements

For NAD$^+$ measurements, 0.5×10^{-6} cells of differentiated or undifferentiated 3T3-L1 cells were pelleted, and total NAD$^+$ was measured using the EnzyChrom NAD$^+$/NADH Assay kit (E2ND-100) from BioAssay Systems (Hayward, CA) according to the manufacturers' instructions.

RNA extraction and real-time PCR analysis

Total RNA from 3T3-L1 cells was extracted using TRIzol (Invitrogen, Carlsbad, CA) with a deoxyribonuclease step. Equal amount of RNA were reverse transcribed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). Real-time PCR was performed using the Rotor-Gene 3000 (Corbett Life Science, now QIAGEN, Valencia, CA). *Cyclophilin* was chosen as the internal control for normalization after screening several candidate genes.

Chromatin immunoprecipitation

ChIP experiments were performed as described earlier (34).

Stable PARP1 knockdown in 3T3-L1 cells

Generation of viruses and transduction of cells was done as described earlier (35). Briefly, pRDI vector expressing PARP1 shRNA was used to transduce 3T3-L1 cells. The shRNA was directed against the sequence encoding the catalytic region of PARP1, which was amplified with the following primers: 5′-GATCCCCAAGAGCGACGCTTATTACTGTTTCAAGAGAACAGTAATAAGCGTCGCTCTTTTTTTGGAAA-3′ (sense) and 5′-AGCTTTTCCAAAAAAAGAGCGACGCTTATTACTGTTCTCTTGAAACAGTAATAAGCGTCGCTCTTGGG-3′ (antisense). Transduced cells were selected through puromycin-resistance gene.

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